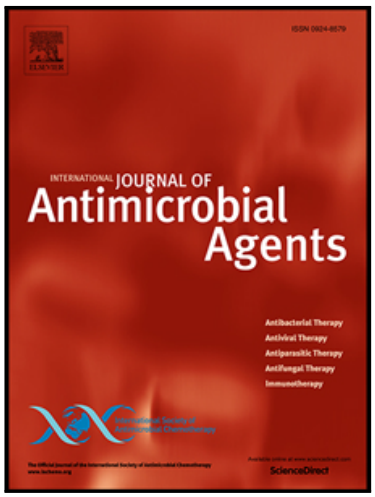

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A Novel *VanA*-Carrying Plasmid in a Clinical Isolate of *Enterococcus avium*

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Running title: novel *vanA*-carrying plasmid

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ABSTRACT

Objectives. Vancomycin resistance among enterococci represents an important medical challenge. This phenotype is frequently associated to the expression of the *vanA* trait and is usually observed in *Enterococcus faecalis/faecium*. In contrast, the VanA phenotype has been very rarely reported in *Enterococcus avium*. *VanA* mobilization is enabled mainly by transposition of Tn*1546* which displays structural variability. Here, we aimed to explore in detail the whole-genome of a vancomycin-resistant *E. avium* isolate (LC0559/18).

Methods. LC0559/18 was recovered from a rectal swab of an ICU-patient hospitalized in Northern Italy. Vancomycin-resistant enterococci were searched with selective plates. Species identification and antimicrobial resistance profile were assessed by MALDI-TOF/MS and VITEK2, respectively. Whole-genome sequencing (WGS) was obtained merging data of both HiSeq (Illumina) and MinION (Oxford Nanopore) platforms.

Results. LC0559/18 showed MICs >16 mg/L and 16 mg/L for vancomycin and teicoplanin, respectively. WGS analysis indicated the presence of two plasmids. pLC0559/18-1 (40’456bp) was of replicon type rep17 and harbored *vanA*, *aph(3’)III*, *ant(6)-Ia* antimicrobial resistance genes (ARGs). The *vanA* cassette was on a Tn*1546-B2* variant reported to date only in *E. faecium*. This variant includes presence of element IS*1216*, as well as absence of transposase ORF1 and resolvase ORF2 of the native Tn*1546*. Moreover, Tn*1546-B2* of LC0559/18 was carried by a novel plasmid backbone. pLC0559/18-2 (52’156bp) was repUS7 positive and did not harbor ARGs.

Conclusions. We report a new plasmid backbone of rarely reported vancomycin-resistant *E. avium* and provide detailed characteristics of its *vanA* genetic environment. In turn, this highlights further mobilization of Tn*1546* among this genus.

KEY WORDS: vancomycin, WGS, VanA, *Enterococcus avium*, Tn*1546*, plasmid

Sir,

Since the first isolation (1986), vancomycin-resistant enterococci (VRE) have been increasingly reported representing nowadays a global public health concern. This is mainly due to their ongoing spread in nosocomial settings along with scarce number of available effective antimicrobials [1, 2]. Among the eight types responsible for acquired vancomycin resistance, the VanA phenotype (defined by high-level vancomycin and teicoplanin resistance) is the best characterized and it counts for most of glycopeptide resistance dissemination in clinical settings [1, 3].

The main indicted for the ongoing spread of vancomycin resistance in clinical isolates of *Enterococcus* spp. is the plastic antimicrobial resistance transposon Tn*1546* (a 10'851bp derivative of Tn*3*, originally described in *E. faecium*). This element is able to easily transpose into diverse conjugative plasmid's backbones and it carries the *vanA* operon responsible for the resistance phenotype [3, 4]. Tn*1546* also shows a typical high degree of heterogeneity illustrated by tens of mutational derivatives of the *A1* transposon prototype. These are distinguished by numerous point mutations, deletions, as well as insertion sequences (ISs) (e.g., IS*1216*) in different positions and orientations, likely acquired by independent events of recombination [1]. However, though frequently described in *E. faecium* and *E. faecalis*, the *vanA* determinant has been rarely described in *E. avium* (Suppl. Table 1). In particular, only one 80kb plasmid carrying a Tn*1546* has been deposited so far [5].

In this work, we describe the first whole-genome of an *E. avium* clinical isolate of VanA phenotype harboring a novel plasmid backbone and carrying a variant of Tn*1546* previously reported only in *E. faecium*.

The isolate (LC0559/18) was recovered from a 26-year-old male patient hospitalized at the intensive care unit (ICU) of the A. Manzoni Hospital (Lecco, Italy) on February 2018 for a distress respiratory syndrome. The patient came from a neurological rehabilitation unit and was already in treatment with ceftriaxone (1 g b.i.d.). On ICU admission, therapy was switched to meropenem (2 g t.i.d.), vancomycin (2 g q.d.), and oseltamivir (150 mg loading dose, then 75 mg b.i.d.) for a 9-day

period. Five days after admission, the patient was investigated by a rectal swab for the presence of VRE within a routine screening program. Culture on selective plates (CHROMID VRE, bioMérieux) was positive for enterococci able to grow in presence of vancomycin. MALDI-TOF/MS (Vitek MS; bioMérieux) allowed identification of *E. avium*. Resistance phenotype was assessed by using the VITEK2 (bioMérieux) and revealed the following antibiotic phenotype (MICs, mg/L): ampicillin (≤2 mg/L), levofloxacin (0.25 mg/L), vancomycin (≥16 mg/L), teicoplanin (16 mg/L), and linezolid (2 mg/L) (EUCAST, version 8.0, 2018).

Hybrid assembly of whole-genome sequencing (WGS) was obtained merging data of both HiSeq (Illumina Inc.) and MinION (Oxford Nanopore) platforms. Briefly, MinION reads underwent trimming with *Porechop* and filtering with *Filtlong* software followed by assembly and contig's circularization by mean of *CANU* and *Circlator* pipelines, respectively. Adapters of HiSeq reads were trimmed implementing *Trimmomatic*, and then further aligned to assemble MinION's contigs using *Bowtie2* aligner. Final genome polishing and annotation were performed using *Pilon* and *Prokka* software. Corrected FASTA sequences (GenBank accession no. **RYZS00000000**) were uploaded on online database Center for Genomic Epidemiology (www.genomicepidemiology.org/) to obtain data on resistance genes, major plasmid's replicon types, and virulence factors. Species identity was confirmed by nucleotide comparison with 16S ribosomal RNA gene of *E. avium* strain ATCC14025 (GenBank accession no. **DQ411811**).

WGS analysis indicated that LC0559/18 carried two plasmids (pLC0559/18-1 of 40'456bp and pLC0559/18-2 of 52'156bp). pLC0559/18-1 was of replicon type rep17, harbored *vanA* (on transposon Tn*1546-B2*-type), *aph(3')III*, and *ant(6)-Ia* antimicrobial resistance genes (ARGs), while pLC0559/18-2 was of replicon type repUS7 and did not carry ARGs. Virulence factors were not present.

Local alignment of complete sequence of pLC0559/18-1 with NCBI Blast indicated that pLC0559/18-1 was a novel plasmid. However, the genetic environment of *vanA*, spanning from 1'909bp to 10'041bp (Figure 1), showed 99% nucleotide identity with 20 deposited sequences of *E.*

faecium, 9 of which covering up to 17kb of its sequence. Part of this region constitutes a variant of the widely reported transposon Tn*1546-B2*-type whose characteristics are the presence of transposase IS*1216* (belonging to IS*6* family) upstream and in opposite orientation with respect to *vanR*, as also the consequent absence of transposase ORF1 and resolvase ORF2 [1, 3]. The present transposon variant (reported to date only in *E. faecium*) is characterized by an additional IS*1215*-like element inserted upstream of *vanS* (Figure 1). The two further additional resistance genes *aph(3')III* and *ant(6)-Ia* were located 1'246bp and 3'146bp upstream of the accessory gene *vanR*, respectively.

The present report exposes detailed characteristics of the *vanA* gene cassette in rarely reported vancomycin-resistant *E. avium*, thereby highlighting variability of Tn*1546* as also its host range's expansion for the VanA phenotype. Moreover, it implicates presence of an emerging and additional bacterial player in the dissemination and evolution of vancomycin resistance in the nosocomial setting [1, 3].

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None

DECLARATIONS

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Competing Interests: None

Ethical Approval: Not required

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LEGEND TO THE FIGURE

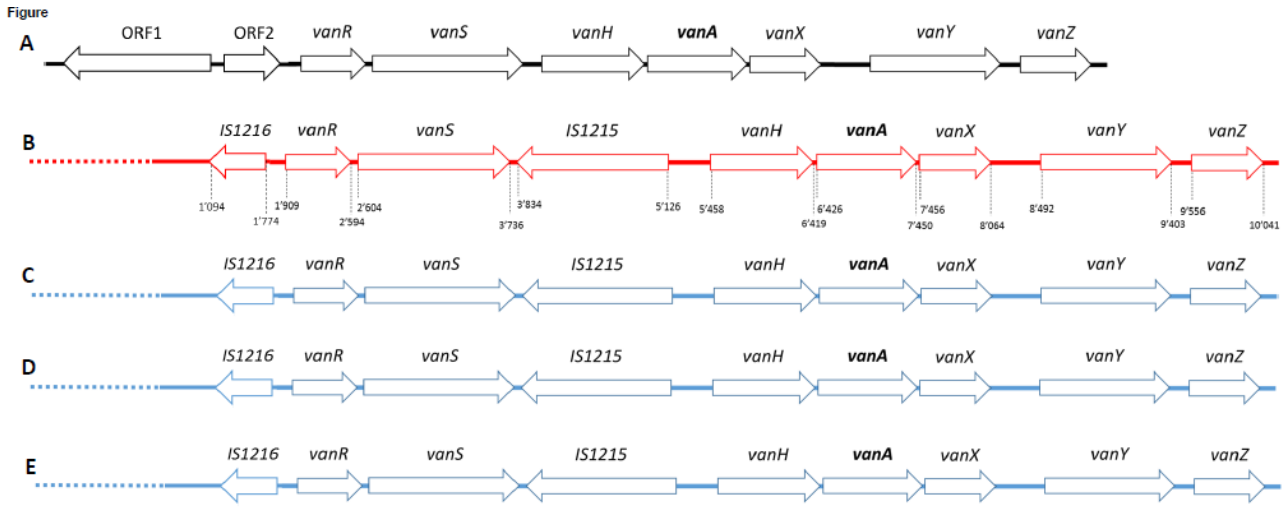

Figure 1: Schematic representation of native and *B2*-type Tn*1546* in pLC055/18-1 and in three other deposited plasmids of *E. faecium*

Numbers indicate starts and ends of depicted genes and are expressed in bp. Arrows indicate directions of the open reading frames (ORFs). ORFs: ORF1, transposase; ORF2, resolvase; IS*1216*, transposase; *VanR*, response regulator; *VanS*, sensor histidine kinase; CDS, IS*1215*-like transposase; *VanH*, pyruvate dehydrogenase; *VanA*, D-Ala-d-Lac ligase; *VanX*, D-Ala-d-Ala dipeptidase; *VanY*, carboxypeptidase; *VanZ*, teicoplanin resistant protein.

A. *Enterococcus faecium* strain BM4147 plasmid pIP816; accession no. KX976485 (deposited on 12.09.2017; France)

B. *Enterococcus avium* strain LC 0559/18 plasmid pLC0559/18-1; present work (GenBank accession no. **RYZS00000000**).

C. *Enterococcus faecium* strain VREfm1 plasmid pPEC286; accession no. KY595962.1 (deposited on 24.5.2018; Hungary).

D. *Enterococcus faecium* strain ISMMS_VRE_10 plasmid ISMMS_VRE10_p3; accession no. CP012474.1 (deposited on 22.03.2017; USA).

E. *Enterococcus faecium* strain Efm008 plasmid pJEG040; accession no. KX810025.1 (deposited on 19.11.16; Australia).